

Listing of Claims

What is claimed is:

1-21. (Canceled)

22. (Currently amended) A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising ~~expressing the nucleic acid of a host cell comprising:~~
culturing a host cell comprising a nucleic acid, the nucleic acid comprising:

~~a nucleic acid sequence comprising:~~

~~P_x-S_x-B_n-(Z_R)-Hir(As_mR)-protein(Y)-T~~

P_x-S_x-B_n-(Z_R)-Hir(As_mR)-protein(Y)-T

where

P_x is a promoter sequence;

S_x is a nucleic acid encoding a signal sequence or leader sequence;

~~B_n~~ B_n is a chemical bond or a codon, wherein n=0-15; B_n being a chemical bond when n=0 and B_n being 1-15 codons[[,]] when n=1-15, respectively is an integer from 1 to 15, or a chemical bond, when n=0;

Z is a codon for lysine or arginine;

R in Z_R is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

~~As_m~~ As_m is a chemical bond or codon, wherein m=0-10; As_m is a chemical bond when m=0, or and As_m is 1-10 codons[[,]] when m=1-10, respectively is an integer from 1 to 10;

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; R in As_m R is an arginine codon or a chemical bond; and

T is an untranslated expression-enhancing nucleic acid sequence,

wherein the host cell comprises the nucleic acid as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally wherein expressing the nucleic acid in the host cell results in formation of the fusion protein in a fermentation supernatant of a culture of the host cell ~~to form the fusion protein in a fermentation supernatant of a cell culture~~ and

isolating said fusion protein from said fermentation supernatant,

wherein said isolating the fusion protein comprises adjusting the pH of said fermentation supernatant to about 2.5 to 3.5 to precipitate non-desired proteins and form a precipitation supernatant and isolating the fusion protein from said precipitation supernatant.

23. (Currently amended) A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising:

~~expressing the nucleic acid of a host cell comprising:~~

(A) culturing a host cell comprising a nucleic acid, the nucleic acid comprising:

~~a nucleic acid sequence comprising:~~

~~Px-Sx-B_n-(ZR)-Hir(As_mR)-protein(Y)-T~~

Px-Sx-B_n-(ZR)-Hir(As_mR)-protein(Y)-T

where

Px is a promoter sequence;

Sx is a nucleic acid encoding a signal sequence or leader sequence;

~~B_n B_n is a chemical bond or a codon, wherein n=0-15; B_n being a chemical bond when n=0 and B_n being 1-15 codons[[,]] when n=1-15, respectively is an integer from 1 to 15, or a chemical bond, when n=0;~~

Z is a codon for lysine or arginine;

R in ZR is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

~~As_m As_m is a chemical bond or codon, wherein m=0-10; As_m is a chemical bond when m=0, or and As_m is 1-10 codons[[,]] when m=1-10, respectively is an integer from 1 to 10; R in As_m R is an arginine codon or a chemical bond;~~

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; and

T is an untranslated expression-enhancing nucleic acid sequence,

wherein the host cell comprises the nucleic acid as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally wherein expressing the nucleic acid in the host cell results in formation of the fusion protein in a fermentation supernatant of a culture of the host cell to form the fusion protein in a fermentation supernatant of a cell culture and

~~(A)~~ separating the fermentation supernatant from the host cell;

~~(B)~~ culturing the host cell in fresh medium;

~~(C)~~ separating the resulting supernatant from the host cell;

~~(D)~~ repeating (B) and (C) several times; and

(EF) isolating the fusion protein from the aforementioned supernatants by adjusting the pH of said supernatants to about 2.5 to 3.5 to precipitate non-desired proteins and form a precipitation supernatant and isolating the fusion protein from said precipitation supernatant.

24. (Currently amended) A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising ~~expressing the nucleic acid of a host cell comprising:~~
culturing a host cell comprising a nucleic acid, the nucleic acid comprising:

~~a nucleic acid sequence comprising:~~

~~P_x-S_x-B_n-(Z_R)-Hir(As_mR)-protein(Y)-T~~

P_x-S_x-B_n-(Z_R)-Hir(As_mR)-protein(Y)-T

where

P_x is a promoter sequence;

S_x is a nucleic acid encoding a signal sequence or leader sequence;

~~B_n~~ B_n is a chemical bond or a codon, wherein n=0-15; B_n being a chemical bond when n=0 and B_n being 1-15 codons[[,]] when n=1-15, respectively ~~is an integer from 1 to 15, or a chemical bond, when n=0;~~

Z is a codon for lysine or arginine;

R in Z_R is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

~~As_m~~ As_m is a chemical bond or codon, wherein m=0-10; As_m is a chemical bond when m=0, or and As_m is 1-10 codons[[,]] when m=1-10, respectively ~~is an integer from 1 to 10;~~

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; R in As_mR is an arginine codon or a chemical bond; and

T is an untranslated expression-enhancing nucleic acid sequence,

wherein the host cell comprises the nucleic acid as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally wherein expressing the nucleic acid in the host cell results in formation of the fusion protein in a fermentation supernatant of a culture of the host cell ~~to form the fusion protein in a fermentation supernatant of a cell culture~~ and

isolating said fusion protein from said fermentation supernatant,

wherein the isolating the fusion protein comprises precipitating the fusion protein from the fermentation supernatant, and further comprising releasing the protein encoded by protein(Y) from the fusion protein and concentrating said protein encoded by protein(Y) by

microfiltration, hydrophobic interaction chromatography, ion exchange chromatography, or a combination of such procedures.

25-29. (Canceled)

30. (Currently amended) A process of fermentative production of a hirudin-mini-proinsulin fusion protein comprising ~~expressing the nucleic acid of a host cell comprising:~~
culturing a host cell comprising a nucleic acid, the nucleic acid comprising:
~~a nucleic acid sequence comprising:~~

~~P_x-S_x-B_n-(Z_R)-Hir(As_mR)-protein(Y)-T~~

P_x-S_x-B_n-(Z_R)-Hir(As_mR)-protein(Y)-T

where

P_x is a promoter sequence;

S_x is a nucleic acid encoding a signal sequence or leader sequence;

~~B_n~~ B_n is a chemical bond or a codon, wherein n=0-15; B_n being a chemical bond when n=0 and B_n being 1-15 codons[[,]] when n=1-15, respectively ~~is an integer from 1 to 15, or a chemical bond, when n=0;~~

Z is a codon for lysine or arginine;

R in Z_R is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

~~As_m~~ As_m is a chemical bond or codon, wherein m=0-10; As_m is a chemical bond when m=0, or and As_m is 1-10 codons[[,]] when m=1-10, respectively ~~is an integer from 1 to 10;~~

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; R in As_m R is an arginine codon or a chemical bond; and

T is an untranslated expression-enhancing nucleic acid sequence,

wherein the host cell comprises the nucleic acid as part of the host cell chromosome, as part of a mini-chromosome, or extra-chromosomally wherein expressing the nucleic acid in the host cell results in formation of the fusion protein in a fermentation supernatant of a culture of the host cell ~~to form the fusion protein in a fermentation supernatant of a cell culture;~~

isolating said fusion protein from said fermentation supernatant, and

~~further comprising~~ releasing mini-proinsulin by treating said fusion protein with trypsin and carboxypeptidase B.

31. (Currently amended) A nucleic acid sequence comprising:

~~Px-Sx-Bn-(ZR)-Hir(AsmR)-protein(Y)-T~~

Px-Sx-B_n-(ZR)-Hir(As_mR)-protein(Y)-T

where

Px is a promoter sequence;

Sx is a nucleic acid encoding a signal sequence or leader sequence;

Bn is 1-15 codons, when n is an integer from 1 to 15, or a chemical bond, when n=0;

Z is a codon for lysine or arginine;

R in ZR is an arginine codon or a chemical bond;

Hir is a nucleic acid sequence encoding hirudin or lepirudin;

~~(AsmR)~~ (As_mR), ~~taken together~~, is an arginine codon or encodes SEQ ID NO:12 (Gly-Asn-Ser-Ala-Arg);

protein(Y) is a nucleic acid sequence encoding mini-proinsulin; and

T is an untranslated expression-enhancing nucleic acid sequence.

32. (Currently amended) The nucleic acid sequence of claim 31 in which ~~(AsmR), taken together~~, encodes SEQ ID NO: 12 (Gly-Asn-Ser-Ala-Arg).

33. (Currently amended) The nucleic acid sequence of claim 31 in which Px is a yeast ADH2 promoter, Sx is an ~~α~~ α factor leader sequence, and Hir encodes hirudin or lepirudin, ~~(AsmR) taken together~~, ~~is either an arginine codon or encodes SEQ ID NO:12 (Gly-Asn-Ser-Ala-Arg)~~.

34. (Canceled)

35. (Currently amended) The nucleic acid of Claim 31 wherein Hir is a nucleic acid sequence encoding lepirudin ~~which has been prepared recombinantly~~.